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Vulnerability of *Asterionella formosa* to *Daphnia* grazing: impact of a fungal parasite

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Introduction

Phytoplankton cells are exposed to zooplankton grazing. Particle size (cell or colony size) of phytoplankton species is an important attribute that determines vulnerability to zooplankton grazing since the range of food particle size of zooplankton like *Daphnia* is limited (GELLER & MÜLLER 1981, STERNER 1989). The freshwater pennate diatom *Asterionella formosa* Hass. forms multicellular colonies that change in size (both in diameter and number of cells per colony) with the seasons (LUND et al. 1963, BELLINGER 1977, HAYAKAWA et al. 1994). Large colonies of *A. formosa* (8 cells colony^{-1}) are known to be less vulnerable to *Daphnia* grazing (LEHMAN & SANDGREN 1985, KNISELY & GELLER 1986, KAGAMI et al. 2004); therefore, the vulnerability of *A. formosa* to grazers may also vary during the seasons. Changes in colony size are triggered by nutrient limitation and temperature (TILMAN et al. 1976, HAYAKAWA et al. 1994), factors that regulate colony size by influencing metabolic processes associated with connection between cells that make up the colony such as the excretion of mucilage pads (HAYAKAWA et al. 1994).

Parasitic chytrids *Zygorhizidium planktonicum* and *Rhizophydium planktonicum* infect *A. formosa* and regulate population dynamics (VAN DONK & RINGELBERG 1983, IBELINGS et al. 2004). Fungal infection affects the metabolic processes of host cells, such as lowering the primary production of *A. formosa* (IBELINGS et al. 2004); therefore, we postulate that fungal infection may affect colony size of *A. formosa* and its vulnerability to zooplankton grazing through its effect on metabolic processes in *A. formosa* cells.

We tested two contrasting hypotheses: The first states that fungal infection makes *A. formosa* more vulnerable to zooplankton grazing. If fungal infection weakens cell-to-cell connections in the colony and breaks up *A. formosa* colonies into smaller fragments, infected *A. formosa* would be more vulnerable to zooplankton grazing due to decrease in colony size. In addition, handling of *A. formosa* by *Daphnia* may break up colonies especially when the cell-to-cell connection is weakened by the fungal parasite. The second opposing hypothesis is that fungal infection makes *A. formosa* colonies less vulnerable to *Daphnia* grazing. In general, highly infected colonies of *A. formosa* tend to aggregate and produce large clumps (KAGAMI personal observation), making particle size bigger, which would make *A. formosa* less vulnerable to zooplankton grazing. In addition, *Daphnia* may facilitate clumping of *A. formosa* colonies, since *Daphnia* is know to induce aggregate formation of the desmid *Staurastrum* (WILTSHIRE et al. 2003).

To test these hypotheses we conducted laboratory experiments using two sizes of *A. formosa* strains. Strain MS03301-1 is small in size and potentially grazed by *Daphnia*. The other strain, MS07702-5, is larger and likely less vulnerable to *Daphnia* grazing.

Key words: Fungal parasitism, *Daphnia*, grazing, Colony size, *Asterionella formosa*

Methods

Two types of *A. formosa* strains were used in the experiment, both isolated from Lake Maarsseveen in different years, MS03301-1 was isolated in 2001 and MS07702-5 in 2002. Both strains were maintained in non-axenic batch cultures with modified Chu-10 medium (KAGAMI et al. 2004). Although MS03301-1 was originally a large strain (average cell size 35 μm, colony size 70 μm) and less vulnerable to *Daphnia* grazing in 2002 (KAGAMI et al. 2004), its cell and colony size became smaller (average cell size 23 μm, colony size 46 μm in 2003) over time. MS07702-5 (average cell size 48 μm, colony size 96 μm) was larger than MS03301-1 in 2003. In this paper, we refer to MS03301-1 as the "small strain" and MS07702-5 as the "large strain".

The chytrid fungus *Zygorhizidium planktonicum* was isolated from Lake Maarsseveen during 2002 (strain FMS04902) and maintained with two types of *A. formosa* host strains (strains MS03301-1 and MS07702-5) in non-axenic culture. *Daphnia galeata*

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hyalina was isolated from Lake Maarsseveen in 1992 and was maintained (KAGAMI et al. 2004).

Six 100-ml flasks were inoculated with 50ml of uninfected *A. formosa* cells (small strain) at a density of 20,000 cells ml^{-1}. Another six 100-ml flasks were inoculated with 50 ml of *A. formosa* cells infected by *Z. planktonicum* (60% of the cells were infected), again at a density of 20,000 cells ml^{-1}. *D. galeata hyalina* was added (3 individuals, 1.69 ± 0.09 μg DW ml^{-1}) to half the flasks of each treatment to serve as *Daphnia* treatments. The other half of the flasks served as control treatments. The same procedure was followed for the large *A. formosa* strain.

All flasks were incubated for 2 days at an irradiance of 40 μmol quanta m^{-2} s^{-1} with a 14:10 h light:dark cycle and a temperature of 18 °C. The flasks were shaken manually twice a day. At the end of the incubation period, the number of *A. formosa* cells and the number of cells per colony were counted. Growth rates (μ: day^{-1}) of *A. formosa* were estimated assuming exponential growth. Grazing rates of *D. galeata hyalina* on infected and uninfected *A. formosa* were calculated from the difference in the growth rates between the control and the *Daphnia* treatments. Effects of *Daphnia* grazing and fungal infection on net growth rates and number of cells per colony of *A. formosa* were assessed by two-way factorial ANOVA independently for small and large strains. The difference in grazing rates of *D. galeata hyalina* between infected and uninfected culture was assessed by *t*-test.

Results

Large strain: The number of cells per colony of the large *A. formosa* strain did not differ significantly between treatments (Fig. 1). Infected cultures aggregated in both control and *Daphnia* treatments. Net growth rates of infected *A. formosa* cultures are significantly lower than those of uninfected culture in both control and *Daphnia* treatments (Fig. 1). Between control and *Daphnia* treatments, net growth rate of uninfected *A. formosa* culture did not differ significantly. Results were the same for infected *A. formosa* cultures. ANOVA showed the effect of fungal infection on net growth rates was significant ($F_{1,12}$ = 181.86, P < 0.0001), but neither effects of *Daphnia* grazing ($F_{1,12}$ = 0.586, P =

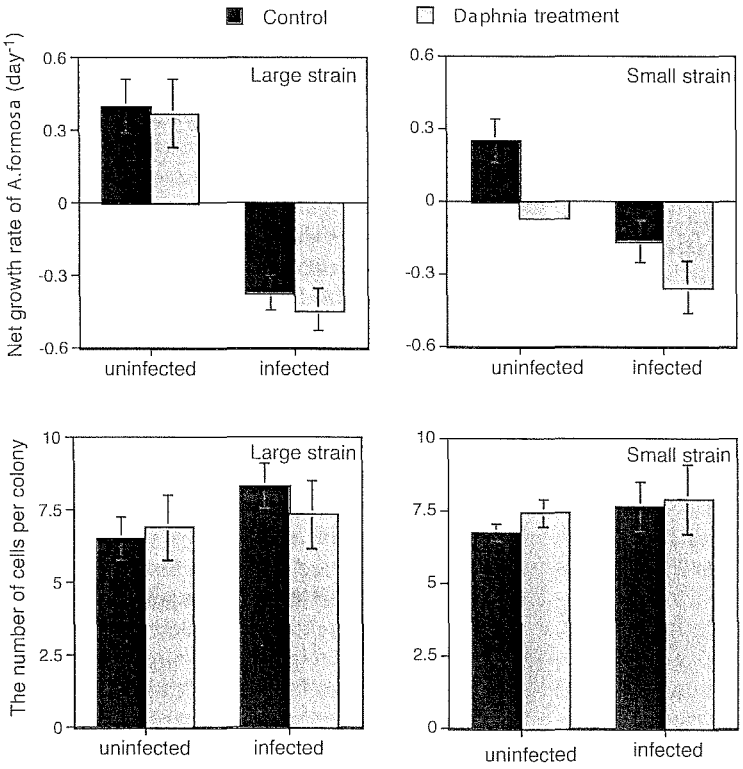

Fig. 1. The net growth rate (upper) and the number of cells per colony (lower) of uninfected and infected *A. formosa* cultures of a large strain (MS07702-5) and a small strain (MS03301-1) in control (black) and *Daphnia* (grey) treatments.

0.4617) nor effects of *Daphnia* grazing × fungal infection interaction ($F_{1,12} = 0.149$, $P = 0.7074$) on net growth rates were significant. These data indicate that *Daphnia* did not affect net growth rates of *A. formosa,* and effects of *Daphnia* grazing did not differ between uninfected and infected cultures.

Small strain: Number of cells per colony of the small *A. formosa* strain did not differ significantly between treatments (Fig. 1). Infected cultures showed aggregations in both control and *Daphnia* treatments. Net growth rates of infected *A. formosa* cultures were significantly lower than those of uninfected cultures (Fig. 1). Comparing net growth rates between control and *Daphnia* treatments, the net growth rate of the uninfected *A. formosa* culture was lower in the *Daphnia* treatment than in the control. The infected culture showed the same pattern (i.e. net growth rate was lower in the *Daphnia* treatment than control). The effect of fungal infection ($F_{1,12} = 53.79$, $P < 0.0001$) and *Daphnia* grazing ($F_{1,12} = 27.76$, $P = 0.0008$) on the net growth rate of *A. formosa* were significant, but effects of fungal infection × *Daphnia* grazing interactions were not significant ($F_{1,12} = 1.873$, $P = 0.2084$), indicating that *Daphnia* grazing did affect net growth rates of *A. formosa*, but did not differ significantly between infected and uninfected cultures.

Grazing rates: Grazing rates of *D. galeata hyalina* on both infected and uninfected cultures were lower on large strain than small strain (Fig. 2). There was no significant difference in grazing rates on large strain between infected and uninfected cultures ($T = 6.580$, $P = 0.5743$). Grazing rates on small strain appeared to be higher in the uninfected culture than in the infected culture, but the difference was not significant ($T = 1.914$, $P = 0.084$).

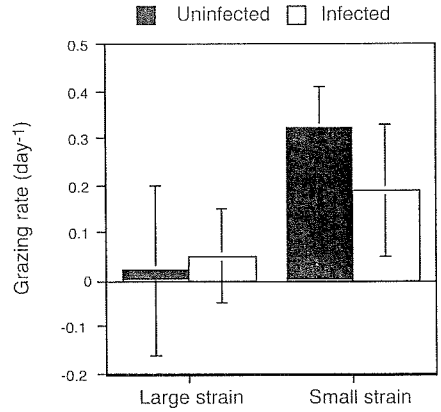

Fig. 2. Grazing rate of *D. galeata hyalina* on uninfected and infected *A. formosa* cultures of a large strain (MS07702-5) and a small strain (MS03301-1).

Discussion

Our experiments showed the small strain of *A. formosa* was grazed by *D. galeata hyalina,* but the large strain was less vulnerable to *D. galeata hyalina* grazing (Fig. 2). For the large strain, grazing rates of *D. galeata hyalina* on *A. formosa* did not change between uninfected and infected cultures, indicating the large strain remained less vulnerable to grazing, even if infected by fungi. The average diameter of the colony was 96 μm, which is larger than the preferred range of food particle size for *D. galeata hyalina* (GELLER & MÜLLER 1981, STERNER 1989). Because the number of cells per colony of infected cultures remained as large as in uninfected cultures (average 7.4 cells per colony, Fig. 1), fungal infection did not change the vulnerability of *A. formosa* to grazing. In addition, feeding activity of *D. galeata hyalina* did not break up the colonies of *A. formosa* into smaller fragments, and *A. formosa* remained less vulnerable to *D. galeata hyalina* grazing. Because the average diameter of the small strain colony was 46 μm, which is within the preferred range of food particle size for *D. galeata hyalina* (GELLER & MÜLLER 1981, STERNER 1989), it was grazed even when colonies consisted of as many as 7.3 cells per colony. Grazing rates of *D. galeata hyalina* on the infected *A. formosa* cultures were slightly lower than those on uninfected cultures, although the difference was not significant (Fig. 2). Because colonies in the infected cultures aggregated, and large clumps are known to be less vulnerable to *Daphnia* grazing (WILTSHIRE et al. 2003), the infected culture must have been less vulnerable to *Daphnia* grazing. Aggregation of colonies must have been induced by fungal infection irrespective of the presence of *Daphnia*, since large clumps were observed in infected

cultures in controls as well as in the *Daphnia* treatment.

The number of cells per colony of *A. formosa* is known to be regulated by nutrient limitation and temperature through metabolic processes associated with cell-to-cell connection (TILMAN et al. 1976, HAYAKAWA et al. 1994). The cells of *A. formosa* are connected by mucilage (adhesive) pads excreted from cells at the apex (ROSOWSKI et al. 1977, HAYAKAWA et al. 1994). A decrease in photosynthetic rate of *A. formosa* has been suggested to be instrumental in reducing colony size, through reduced production of mucilage and/or changes in chemical composition of the pads (HAYAKAWA et al. 1994). Although fungal infection affects metabolic processes of host cells and lowers production rate (IBELINGS et al. 2004), our experiments showed that fungal infection did not change colony size (the number of cells per colony) of host cells. Fungal infection might not have any influence on mucilage excretion that connects cells, probably because the fungi infected their host cells after colonies were formed, and infected cells produce few new daughter cells since almost every infection kills the host cell.

Fungal infection may affect sinking rates of host cells. Sinking rate of *A. formosa* is influenced by morphology of colonies and physiological condition of cells. The stellate form of colonies decreases sinking velocity (JAWORSKI et al. 1988). Cells in the stationary growth phase, or dead cells, sink faster than exponentially growing cells (TILTMAN & KILHAM 1976). Although fungal infection did not affect the number of cells per colony or the stellate form of the colony (Fig. 1), it did change the physiological condition of cells and lead to death of host cells. Furthermore, fungal infection induced the aggregate formation of colonies in our experiments, which makes particle size bigger and increases sinking velocity (TILTMAN & KILHAM 1976). Thus, fungal infection would make host cells sink faster. Enhanced sedimentation of *A. formosa* blooms will affect material cycling in lakes. Although nutrients within *A. formosa* cells are consumed by fungi, which in turn are grazed by *Daphnia* (KAGAMI et al. 2004), silicate or other materials of the frustule of *A. formosa* would hardly be grazed and will be transported to the bottom of the lakes. Thus, fungal infection may make a difference in material cycling between nutrients within host cells and those in frustule of host cells such as silicate.

In conclusion, the present study showed that fungal infection did not make *A. formosa* more vulnerable to *Daphnia* grazing. Fungal infection did, however, induce aggregation of colonies, which seemed to make *A. formosa* somewhat less susceptible to *Daphnia* grazing and may make *A. formosa* sink faster.

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